

Comparison of the Antibacterial Effects of Nanosil, Chlorhexidine and Probiotic Mouthwashes on Periodontal Pathogens

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Objectives Periodontal diseases are one of the common oral diseases and microbial oral flora is one major factor responsible for it. Elimination of periodontal pathogens is particularly important in managing the periodontal state. This study aimed to assess the antibacterial effects of Nanosil, chlorhexidine (CHX) and probiotic mouthwashes on periodontal pathogens.

Methods In this in-vitro study, the bacteria (*A. actinomycetemcomitans*, *P. aeruginosa*, *K. pneumonia*, *E. coli*, *S. aureus*, *B. cereus*, *B. subtilis*, *S. typhimurium*, *E. coli* O-157 and *E. coli* PTCC 1338) were cultured using specific culture media. Microbial suspension was prepared by dissolving 1 or 2 microbial colonies in tryptic soy broth. Nanosil, CHX and probiotic mouthwashes were added to the wells containing bacterial suspension. Samples were taken from wells showing turbidity and cultured in plates. The minimum inhibitory concentration (MIC) of mouthwashes was determined by repeated measuring of the growth inhibition zones. Kolmogorov-Smirnov test, One-way ANOVA, and Scheffe's post hoc was used for statistical analysis.

Results Probiotic mouthwash had greater antibacterial effects than other mouthwashes and caused larger growth inhibition zones. For *S. aureus* and *S. typhimurium*, the mean diameter of the growth inhibition zone was not significant ($p > 0.05$), while for other tested bacteria were significant ($p < 0.05$).

Conclusion Probiotic mouthwash decreases the pathogenic oral flora and stabilizes the beneficial flora in oral cavity.

Keywords Probiotic, Chlorhexidine, Mouthwash, Periodontal Disease

Introduction

Periodontal diseases are among the most important inflammatory diseases with a multifactorial etiology affecting the tooth supporting structures. Imbalance in the oral microbial flora is a major factor responsible for development of periodontal disease¹.

The most important signs and symptoms of periodontal disease are related to an increase in count of *P. gingivalis*, *T. forsythia*, *T. denticola* and *A. actinomycetemcomitans*². Elimination of pathogenic species is particularly important in treatment of periodontal diseases while plaque control plays an important role in preventing them^{2,3}. The currently used treatment methods include plaque control, antibiotic therapy and periodontal surgery⁴. Mouthwashes are commonly used for plaque control. Chlorhexidine (CHX) di-gluconate is a prescription mouthwash, which is commonly prescribed for periodontal patients. It is more effective on Gram-positive bacteria compared to Gram-negatives⁵. Clinical studies show oral bacteria developing resistance to CHX in long-term. Also, CHX has many side effects on oral cavity^{5,6}. Nanosil is a mouthwash that has antiseptic properties. Nanosil D1, can be used as an effective mouth rinse for periodontal disease. Nanosil D1 also has bleaching properties since it contains hydrogen peroxide and eliminates dental stains⁷; However, some concerns exist regarding the long-term use of hydrogen peroxide such as risk of tissue injury, proliferation of yeasts

and carcinogenic effects⁸.

Due to the emergence of resistant bacterial strains and the complications of current periodontal treatments; use of probiotic bacteria has increased for treatment of diseases such as in oral conditions. Probiotic bacteria are viable bacteria that efficiently improve the balance of microbial flora⁴. Probiotics have been used for treatment of oral conditions such as caries, gingivitis, halitosis, candidiasis, burning mouth syndrome, xerostomia and periodontal diseases⁹. Many bacterial species are used as probiotics for inhibition and control of oral and gingival inflammation. Studies on the application of probiotics in periodontal therapy are scarce, which may reflect the gap of information on the etiology of disease and preventive factors¹⁰. Numerous mouthwashes are available in the market and their efficacy has not been well studied. Considering the recent introduction of Nanosil and probiotic mouthwashes to the Iranian market, no previous study has evaluated their efficacy. As a result, this study was carried out to assess the antibacterial effects of Nanosil, CHX and probiotic mouthwash on some oral main periopathogens.

Materials and Methods

Microbial sample collection

In this in-vitro experimental study, nine bacterial species (*A. actinomycetemcomitans*, *P. aeruginosa*, *K. pneumonia*, *E. coli*, *S. aureus*, *B. cereus*, *B. subtilis*, *S. typhimurium*, *E.*

coli O-157 and *E. coli* PTCC 1338) were exposed to three mouthwashes (Nanosil, CHX and probiotic mouthwashes). These bacteria were obtained in lyophilized form from the Genetic Engineering and Biotechnology Research Center. These bacteria were cultured in Mueller Hinton agar plates (incubated for 48 hours at 37 degrees celcius). Microorganisms were cultured in brain heart infusion broth and microbial suspension was prepared by dissolving one or two microbial colonies in TSB.

Preparation of probiotic mouthwash

To prepare the probiotic mouthwash, lyophilized form of *L. salivarius* NK01 probiotic bacterium along with maltodextrin powder and phosphate buffered saline solution were used¹¹. Thirty seconds prior to its use on pathogens, the powder and liquid of probiotic mouthwash were mixed and added to the culture medium.

Determination of MIC

Bacteria were cultured in Mueller-Hinton agar plates. A total of 100 μ L of sterile Mueller-Hinton broth was added to wells (number of wells determined by the number and count of bacteria). Three samples of each 3 mouthwashes were in contact with each of the nine bacteria; each test was repeated 3 times making the total number of wells for each mouthwash 81 excluding the positive and negative control wells. Two test tubes were also included as the positive and negative control groups. Serial dilutions of the mouthwashes were prepared in sterile Mueller-Hinton agar medium.

The bacteria were gradually dissolved in saline until reaching 0.5 McFarland turbidity. Diluted bacterial suspension was vortexed again and 100 μ L of it was added to the wells containing the serially diluted mouthwashes and the positive control group. This suspension was not added to the negative control group. It was then incubated for 24 hours at 37 degrees celcius. Concentration of the first well without turbidity due to bacterial growth was considered as MIC. This tube was determined by comparing

the tubes with the negative control tube.

Statistical analysis

Repeated measurements were used to determine the growth inhibition zone of each bacterial strain, then Kolmogorov-Smirnov test was used to assess the normality of growth inhibition zone data. One-way ANOVA and Scheffe's post hoc test was utilized to assess the data significance.

Results

Growth inhibition zones

Eighty one wells were used for each mouthwash. Table 1 shows that out of nine bacterial species evaluated in this study, seven showed significant differences in the diameter of the growth inhibition zones following exposure to the three mouthwashes. *B. subtilis* (p=0.003), *B. cereus* (p=0.002), *E. coli* (PTCC1338) (p=0.041), *K. pneumonia* (p=0.006), *P. aeruginosa* (p=0.003), *E. coli* O-157 (p=0.001) However, *S. aureus* and *S. typhimurium* had similar growth inhibition zones in exposure to the three mouthwashes.

For *S. aureus* and *S. typhimurium*, the mean diameter of the growth inhibition zone was not significant. The growth inhibition zone of *B. subtilis* was significantly different in exposure to mouthwashes. For *B. cereus*, the difference in this regard was significant. In *E. coli* (PTCC 1338), the mean diameter of the growth inhibition zone was statistically significant. In *K. pneumonia*, the mean diameter of the growth inhibition zone was significantly different in exposure to mouthwashes. For *P. aeruginosa*, the difference in this regard was statistically significant. In *E. coli* O-157, the difference in this regard was significant. For *A. actinomycetemcomitans*, Scheffe's post hoc test showed that the mean diameter of the growth inhibition zone in exposure to mouthwash was significant.

Table 1. Comparison of the mean diameter of the growth inhibition zone of different microorganisms exposed to the three mouthwashes

Microorganism	Probiotic growth inhibition zone (mm)	Mouthwashes		P value
		CHX growth inhibition zone (mm)	Nanosil growth inhibition zone (mm)	
<i>S. aureus</i>	11.83 \pm 1.06	10.53 \pm 0.61	10.1 \pm 0.52	0.072
<i>B. subtilis</i>	12.57 \pm 0.81	11.07 \pm 0.30	9.6 \pm 0.52	0.003*
<i>B. cereus</i>	11.37 \pm 1.2	8.5 \pm 0.5	7.4 \pm 0.4	0.002*
<i>E. coli</i> (PTCC1338)	21.67 \pm 1.5	19.53 \pm 1.7	17.3 \pm 0.87	0.041*
<i>S. typhimurium</i>	19.37 \pm 0.70	19.13 \pm 0.85	19.47 \pm 0.45	0.836
<i>K. pneumonia</i>	15.37 \pm 1.2	13.47 \pm 0.50	12 \pm 0.10	0.006*
<i>P. aeruginosa</i>	24.33 \pm 0.57	23.27 \pm 0.64	21.5 \pm 0.5	0.003*
<i>E. coli</i> O-157	21.87 \pm 0.77	18.53 \pm 0.61	18.37 \pm 0.55	0.001*
<i>A. actinomycetemcomitans</i>	23 \pm 1.0	19.27 \pm 0.94	18.37 \pm 0.55	0.001*

* Level of significance using one-way ANOVA

MIC assessment

Table 2 shows that for the nine microorganisms evaluated

in this study, the MIC of probiotic mouthwash was higher than that of the other two mouthwashes. For assessment of

MIC of these pathogenic bacteria 0.2mg/mL concentration of mouthwashes were used.

Table 2- The MIC of the three mouthwashes for different microorganisms tested in this study

Microorganism	MIC		
	Probiotic	Chlorhexidine	Nanosil
<i>S. aureus</i>	512 mg/mL	512mg/mL	256 mg/mL
<i>B. subtilis</i>	64 mg/mL	32 mg/mL	32 mg/mL
<i>B. cereus</i>	32 mg/mL	32 mg/mL	16 mg/mL
<i>E. coli</i> (PTCC1338)	1024 mg/mL	512 mg/mL	256 mg/mL
<i>S. typhimurium</i>	256 mg/mL	256 mg/mL	128 mg/mL
<i>K. pneumonia</i>	64mg/mL	16 mg/mL	16 mg/mL
<i>P. aeruginosa</i>	128mg/mL	64mg/mL	32 mg/mL
<i>E. coli</i> O-157	256mg/mL	128mg/mL	128mg/mL
<i>A.actinomycetemcomitans</i>	512mg/mL	256mg/mL	512mg/mL

Discussion

Presence of probiotics in oral microflora guarantees the success of treatment with probiotics and indicates their compliance with oral ecosystem. In previous studies, the effect of probiotics on periodontal health has not been well elucidated. Preliminary data from different studies indicate the positive effects of probiotics but clinical trials are required to confirm it.

In this study, number of cultured colonies was counted before and after exposure to mouthwashes. The results of this study showed that probiotic mouthwash was more effective in inhibiting microorganisms and yielded larger growth inhibition zones compared to other mouthwashes.

Horster and Korf in 1976 compared the efficacy of CHX and hydrogen peroxide for periodontal prophylaxis in patients with mandibular fractures. The results of their study showed that 0.2% CHX was significantly more effective than hydrogen peroxide for inhibition of plaque formation. Plaque reduction was 69% by CHX and 22% by hydrogen peroxide; in our study, CHX had greater efficacy ($p < 0.05$) for inhibition of microorganisms compared to Nanosil by showing greater diameter of growth inhibition zone¹².

Zelić et al. (2009) compared the effects of CHX and Nanosil on gingival inflammation and showed that CHX was more effective than Nanosil in decreasing plaque index and gingival index and a significant difference existed in this regard between the two mouthwashes but no significant difference was noted between the two in terms of bleeding index¹³. Also, staining of teeth following the use of Nanosil was significantly less than that following the use of CHX. They clinically compared the effects of CHX and Nanosil while the current study had an in vitro design and showed that CHX had stronger antibacterial effects than Nanosil against both aerobic and anaerobic microorganisms.

Nanosil contains hydrogen peroxide, which prevents the proliferation of anaerobic microorganism due to release of oxygen. Gusberti et al. (1988), Moran et al. (1995) and Menendez et al. (2005) evaluated the antibacterial effects of CHX and oxidizing mouthwashes and showed that CHX was more effective than oxidizing mouthwashes¹⁴⁻¹⁶. In the current study, CHX had a stronger antibacterial activity than Nanosil and a weaker antibacterial effect than the

probiotic mouthwash. The current results showed that presence of *L. Salivarius* as a probiotic microorganism can decrease the growth and proliferation of main periodontal pathogens such as *A. actinomycetemcomitans* and major pathogens responsible for internal diseases. This can be attributed to the interaction of bacteria. In fact, probiotics can alter the structure or physiology of pathogenic microorganisms with different mechanisms or prevent the colonization and growth of pathogens.

Mombeili et al. (1994) assessed the presence of *A. actinomycetemcomitans* in periodontitis patients before and after scaling and root planing and found *A. actinomycetemcomitans* in 40% of patients before and 23% of patients after scaling and root planing (SRP). Presence of this microorganism even after debridement of the root surface has been reported¹⁷. This shows that bacteria as one of the main periopathogens is resistant to SRP treatments; however, in our study probiotic mouthwash could significantly inhibit its growth compared to other mouthwashes; ergo, probiotic mouthwash can be used as an alternative or along with SRP treatment to reduce *A. actinomycetemcomitans*. Vivekananda et al. (2010) used *L. reuteri* as a probiotic microorganism and found that it produced 3 hydroxypropionaldehyde antimicrobial agent, which explains anti-pathogenic effect of this microorganism¹⁰. However, the mechanism behind the anti-pathogenicity of *L. salivarius* NK01 probiotic microorganism has not been well evaluated in the molecular level. In agreement with this finding, two randomized controlled trials in Japan evaluated the effect of probiotics on periodontal pathogens. Ishihawa et al. (2003) and Matsuoka et al (2006) reported that oral intake of probiotic tablets containing *L. salivarius* by healthy volunteers decreased *P. gingivalis* count in subgingival plaque and saliva^{18,19}.

Koll-klais et al, in 2005 showed that presence of heterofermentative lactobacilli such as *L. salivarius* was very effective against periodontal pathogens in chronic periodontitis patients. Koll et al. (2006) isolated 22 lactobacillus strains from the mouth and showed that most lactobacillus strains inhibited *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans*. This revealed the ability of oral lactobacilli in playing a probiotic role for maintaining periodontal health²⁰.

The current results agreed with those of Krasse et al. They assessed the efficacy of *L. reuteri* in decreasing gingivitis and plaque index and showed that *L. reuteri* probiotic microorganism was greatly effective in decreasing gingivitis and plaque index in patients with moderate to severe gingivitis²¹. Similarly, Vivekananda et al. showed that *L. reuteri* probiotic mouthwash significantly decreased bleeding on probing when compared to scaling and root planing alone (reduction of bleeding on probing by 35% after 42 days)¹⁰. Ma et al. demonstrated the effect of *L. reuteri* probiotic microorganism on human epithelial cells and reported that *L. reuteri* inhibited the release of IL-8 and tumor necrosis factor- α ²². Twetman et al. (2009) evaluated the efficacy of probiotic chewing gum for decreasing gingival inflammation and level of inflammatory mediators in gingival crevicular fluid in patients with gingivitis. They noticed that probiotics decreased IL-8, tumor necrosis factor- α , and IL-1 β in gingival crevicular fluid and reduced inflammation; these results confirmed the current findings²³). This study confirmed the antibacterial effects of *L. salivarius* NK01; thus, *L. salivarius* NK01 is recommended as an adjunct to scaling and root planing for prevention of periodontal disease and in the maintenance phase of periodontal therapy. Probiotics are capable of adhering to surfaces and balancing the replacement of pathogenic microorganisms with non-pathogenic strains. Future studies and clinical trials are required to find stronger evidence confirming these findings.

Conclusion

Although CHX can be prescribed for a short period of time as an adjunct for plaque control, it is not recommended for long-term use because it causes tooth discoloration. This study showed that Nanosil mouthwash was less efficient than that of CHX regarding antibacterial effects; however, future studies with larger sample sizes are required to assess the efficacy of Nanosil in longer periods of time. Probiotic NK01 mouthwash showed more inhibition growth diameter than Nanosil and CHX and thus can be used as a replacement to other mouthwashes with better efficacy; however, future clinical trials are required to confirm this.

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Compliance with Ethical Standards

Ethical approval: All procedures performed in studies were In-Vitro and approved by Zanjan University of Medical Sciences ethical committee.

Conflict of Interests

None Declared ■

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